

What is claimed is:

1. A replication competent polynucleotide comprising:  
a 5' non-translated region (NTR), a 3' NTR, and a first coding sequence present between the 5' NTR and 3' NTR and encoding a hepatitis C virus polyprotein, wherein the polyprotein comprises an isoleucine at about amino acid 2204, and further comprises an adaptive mutation selected from the group of an arginine at about amino acid 1067, an arginine at about amino acid 1691, valine at about amino acid 2080, an isoleucine at about amino acid 1655, an arginine at about amino acid 2040, an arginine at about amino acid 1188, and a combination thereof.
2. The replication competent polynucleotide of claim 1 further comprising a second coding sequence.
3. The replication competent polynucleotide of claim 2 wherein the second coding sequence encodes a marker.
4. The replication competent polynucleotide of claim 2 wherein the second coding sequence encodes a transactivator.
5. The replication competent polynucleotide of claim 1 wherein the 5' NTR, the 3' NTR, and the nucleotide sequence encoding the polyprotein are genotype 1a.
6. The replication competent polynucleotide of claim 1 wherein the hepatitis C virus polyprotein is a subgenomic hepatitis C virus polyprotein.
7. The replication competent polynucleotide of claim 1 wherein the hepatitis C virus polyprotein comprises cleavage products core, E1, E2, P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B.

8. The replication competent polynucleotide of claim 1 further comprising a nucleotide sequence having cis-acting ribozyme activity, wherein the nucleotide sequence is located 3' of the 3' NTR.

9. A replication competent polynucleotide comprising:

a 5' non-translated region (NTR), a 3' NTR, and a first coding sequence present between the 5' NTR and 3' NTR and encoding a hepatitis C virus polyprotein comprising cleavage products core, E1, E2, P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B, wherein the polyprotein comprises an isoleucine at about amino acid 2204, an arginine at about amino acid 1067, and an arginine at about amino acid 1691.

10. The polynucleotide of claim 9 further comprising a second coding sequence.

11. The polynucleotide of claim 10 wherein the second coding sequence encodes a marker .

12. The polynucleotide of claim 10 wherein the second coding sequence encodes a transactivator.

13. The polynucleotide of claim 9 wherein the 5' NTR, polyprotein, and 3' NTR are genotype 1a.

14. A method for making a replication competent polynucleotide comprising:  
providing a polynucleotide comprising a 5' NTR, 3' NTR, a first coding sequence present between the 5' NTR and 3' NTR and encoding a hepatitis C virus polyprotein, wherein the polyprotein comprises a serine at about amino acid 2204, a glutamine at about amino acid 1067, a lysine at about amino acid 1691, a phenylalanine at about amino acid 2080, a valine at about amino acid 1655, a lysine at about amino acid 2040, or a glycine at about amino acid 1188 and wherein the 5' NTR, polyprotein, and 3' NTR are genotype 1a; and  
altering the coding sequence such that the polyprotein encoded thereby comprises an isoleucine at amino acid 2204, and at least one adaptive mutation

selected from the group consisting of an arginine at about amino acid 1067, an arginine at about amino acid 1691, a valine at about amino acid 2080, an isoleucine at about amino acid 1655, an arginine at about amino acid 2040, an arginine at about amino acid 1188, and a combination thereof.

15. The method of claim 14 wherein the hepatitis C virus polyprotein is a subgenomic hepatitis C virus polyprotein.

16. The method of claim 14 wherein the hepatitis C virus polyprotein comprises cleavage products core, E1, E2, P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B.

17. A replication competent polynucleotide produced by the method of claim 14.

18. A method for identifying a compound that inhibits replication of a replication competent polynucleotide, the method comprising:

contacting a cell comprising a replication competent polynucleotide with a compound, the replication competent polynucleotide comprising 5' NTR, 3' NTR, a first coding sequence present between the 5' NTR and 3' NTR and encoding a hepatitis C virus polyprotein, wherein the hepatitis C virus polyprotein comprises an isoleucine at about amino acid 2204, and further comprises an adaptive mutation selected from the group of an arginine at about amino acid 1067, an arginine at about amino acid 1691, valine at about amino acid 2080, an isoleucine at about amino acid 1655, an arginine at about amino acid 2040, an arginine at about amino acid 1188, and a combination thereof;

incubating the cell under conditions wherein the replication competent polynucleotide replicates in the absence of the compound; and

detecting the replication competent polynucleotide, wherein a decrease of the replication competent HCV polynucleotide in the cell contacted with the compound compared to the replication competent polynucleotide in a cell not contacted with the compound indicates the compound inhibits replication of the replication competent polynucleotide.

19. The method of claim 18 wherein detecting the replication competent polynucleotide comprises nucleic acid amplification.
20. The method of claim 18 wherein the replication competent polynucleotide further comprises a second coding sequence encoding a marker, and wherein detecting the replication competent polynucleotide comprises identifying the marker.
21. The method of claim 18 wherein the replication competent polynucleotide further comprises a second coding sequence encoding a transactivator, wherein the cell comprises a polynucleotide comprising a transactivated coding sequence encoding a detectable marker and an operator sequence operably linked to the transactivated coding sequence, wherein the transactivator interacts with the operator sequence and alters expression of the transactivated coding sequence, and wherein detecting the replication competent polynucleotide in the cell comprises detecting the detectable marker encoded by the transactivated coding sequence.
22. The method of claim 18 wherein the cell is a human hepatoma cell.
23. The method of claim 18 wherein the hepatitis C virus polyprotein is a subgenomic hepatitis C virus polyprotein.
24. The method of claim 18 wherein the hepatitis C virus polyprotein comprises cleavage products core, E1, E2, P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B.
25. The method of claim 18 wherein the 5' NTR, polyprotein, and 3' NTR are genotype 1a.
26. A method for selecting a replication competent polynucleotide, the method comprising:
  - incubating a cell in the presence of a selecting agent, wherein:
    - the cell comprises a polynucleotide comprising a 5' non-translated region (NTR), a 3' NTR, and a first coding sequence present between

the 5' NTR and 3' NTR and encoding a hepatitis C virus polyprotein, and a second coding sequence, wherein the polyprotein comprises an isoleucine at about amino acid 2204, and further comprises an adaptive mutation selected from the group of an arginine at about amino acid 1067, an arginine at about amino acid 1691, valine at about amino acid 2080, an isoleucine at about amino acid 1655, an arginine at about amino acid 2040, an arginine at about amino acid 1188, and a combination thereof;

the second coding sequence encodes a selectable marker conferring resistance to the selecting agent; and

the selecting agent inhibits replication of a cell that does not express the selectable marker; and

detecting a cell that replicates in the presence of the selecting agent, wherein the presence of such a cell indicates the polynucleotide is replication competent.

27. The method of claim 26 wherein the selecting agent is an antibiotic.

28. The method of claim 26 wherein the cell is a human hepatoma cell.

29. The method of claim 26 wherein the cell is a first cell, the method further comprising:

obtaining a virus particle produced by the first cell;

exposing a second cell to the isolated virus particle and incubating the second cell in the presence of the selecting agent; and

detecting a second cell that replicates in the presence of the selecting agent, wherein the presence of such a cell indicates the replication competent polynucleotide in the first cell produces an infectious virus particle.

30. The method of claim 26 wherein the hepatitis C virus polyprotein is a subgenomic hepatitis C virus polyprotein.

31. The method of claim 26 wherein the hepatitis C virus polyprotein comprises cleavage products core, E1, E2, P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B.

32. The method of claim 26 wherein the 5' NTR, polyprotein, and 3' NTR are genotype 1a.

33. A method for detecting a replication competent polynucleotide, the method comprising:

incubating a cell comprising a replication competent polynucleotide, wherein:

the replication competent polynucleotide comprises a 5' non-translated region (NTR), a 3' NTR, and a first coding sequence present between the 5' NTR and 3' NTR and encoding a hepatitis C virus polyprotein, and a second coding sequence encoding a transactivator, wherein the polyprotein comprises an isoleucine at about amino acid 2204, and further comprises an adaptive mutation selected from the group of an arginine at about amino acid 1067, an arginine at about amino acid 1691, valine at about amino acid 2080, an isoleucine at about amino acid 1655, an arginine at about amino acid 2040, an arginine at about amino acid 1188, and a combination thereof;

the cell comprises a transactivated coding region and an operator sequence operably linked to the transactivated coding region; and

the transactivated coding region encodes a detectable marker, wherein the transactivator alters transcription of the transactivated coding region; and

detecting the detectable marker, wherein the presence of the detectable marker indicates the cell comprises a replication competent polynucleotide.

34. The method of claim 33 wherein the hepatitis C virus polyprotein is a subgenomic hepatitis C virus polyprotein.

35. The method of claim 33 wherein the hepatitis C virus polyprotein comprises cleavage products core, E1, E2, P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B.

36. The method of claim 33 wherein the 5' NTR, polyprotein, and 3' NTR are genotype 1a.

37. A kit comprising:

a replication competent polynucleotide comprising a 5' non-translated region (NTR), a 3' NTR, and a first coding sequence present between the 5' NTR and 3' NTR and encoding a hepatitis C virus polyprotein, and a second coding sequence encoding a transactivator, wherein the polyprotein comprises an isoleucine at about amino acid 2204, and further comprises an adaptive mutation selected from the group of an arginine at about amino acid 1067, an arginine at about amino acid 1691, valine at about amino acid 2080, an isoleucine at about amino acid 1655, an arginine at about amino acid 2040, an arginine at about amino acid 1188, and a combination thereof; and

a cell comprising a polynucleotide comprising a transactivated coding sequence encoding a detectable marker and an operator sequence operably linked to the transactivated coding sequence, wherein the transactivator interacts with the operator sequence and alters expression of the transactivated coding sequence.

38. The method of claim 37 wherein the hepatitis C virus polyprotein is a subgenomic hepatitis C virus polyprotein.

39. The method of claim 37 wherein the hepatitis C virus polyprotein comprises cleavage products core, E1, E2, P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B.

40. The method of claim 37 wherein the 5' NTR, polyprotein, and 3' NTR are genotype 1a.